Developing a battery of in vitro assays targeting key molecular initiation events in thyroid hormone regulation to assess Thyroid Hormone-Disrupting Chemicals

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Introduction and aims

Chemicals disrupting the thyroid hormone (TH) system interfere with TH signalling in humans, which can be linked with many human health problems such as neurodevelopmental disorders in children, thyroid neoplasms, autoimmune thyroid disorders and increased cardiovascular risk due to altered lipid metabolism in adults.

Aims: In the EU H2020 ERGO project, we are designing a battery of in vitro assays for evaluating chemicals disrupting thyroid hormone signalling. The project is involved in studies concerned with:

(i) the development and pre-validation of a battery of in vitro methods, which are based on the adverse outcome pathway network concept addressing priority molecular initiation events (MIEs) in the thyroid hormone regulation

(ii) characterization of the potential effects of selected compounds relevant to human exposure to disrupt the thyroid hormone system at different levels

(iii) linking in vitro mechanisms to adverse affects observed in vivo

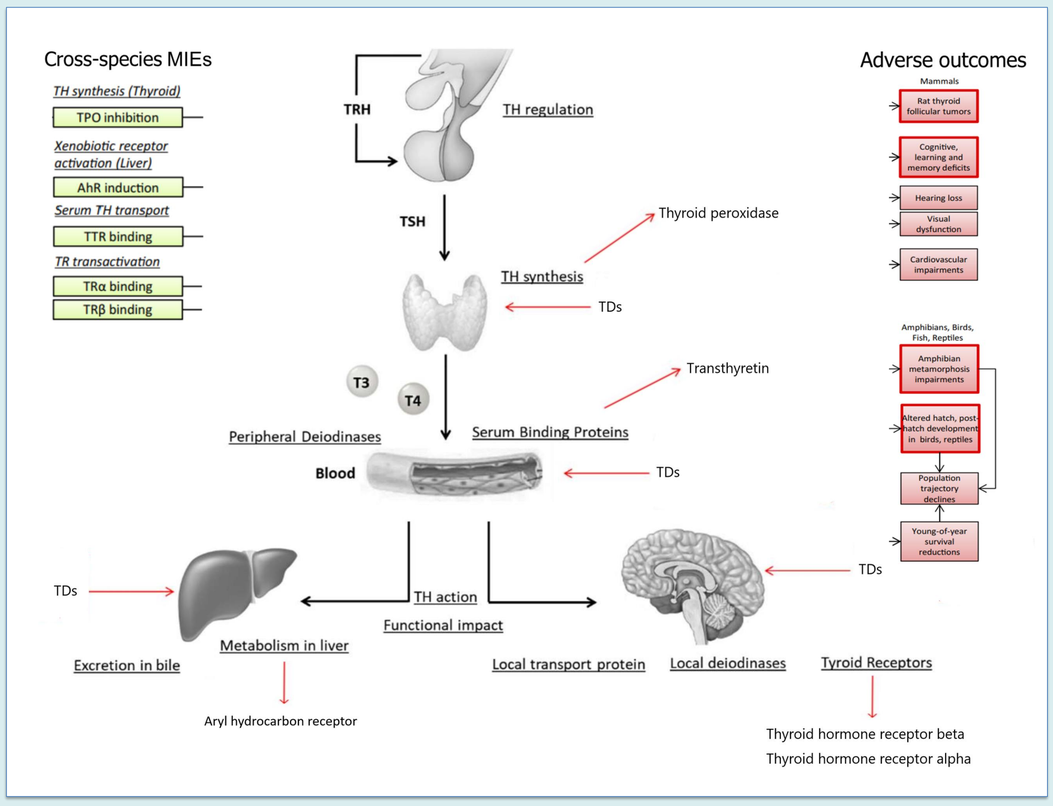

Figure 1 Action of thyroid hormone-disrupting chemicals (TDCs) on the hypothalamic–pituitary–thyroid axis, cross-species relevant molecular Initiating Events (MIEs) and adverse outcomes. The black arrows indicate the endocrine axis, and the red arrows indicate the organs/tissues targeted by the TDCs (Noyes et al., 2019 and Street et al., 2018)

Methods

Several in vitro models are used in assays for the assessment of MIEs for thyroid hormone signalling disruption:

TH metabolisation: AZ-AhR Luciferase reporter cell line is used for the assessment of the interaction of samples with aryl hydrocarbon receptor (AhR), which is a ligand-activated transcriptional factor that regulates genes implicated in chemical metabolism.

TH synthesis: Enzyme inhibition assays for Thyroid peroxidase (TPO) are performed to evaluate the interaction of samples with the activity of the TPO enzyme that is crucial for TH synthesis. Series of models focused on human and rat TPO were established based on the Nthy-ori 3-1 (human) and FRTL-5 (rat) cell lines, HEK293T cell lines transfected with hTPO and rat thyroid microsomes. Novel hTPO overexpressing models were developed and characterized. Several detection methods have been developed and employed for the different models.

TH signalling: PZ-TR Luciferase reporter cell line is used to assess the interaction of chemicals with the TH receptor.

TH transport: thyroxine-transthyretin (T4-TTR) binding assay is performed to examine the effects on TH transport.

Future

(1) Papers about (1) TPO assays and (2) other assays will be published

(2) The final thesis will be written

Figure 2 (a) TPO inhibition assay results in Nthy-ori (human), FRTL-5 (rat) cell lines and three hTPO transfected HEK cell lines-TPOSA1, TPOSA2 and TPOSB; (b) PZ-TR assay results; (c) TTR assay results.

Results

- Established battery of assays, developed and characterized novel *in vitro* models
- ERGO-prioritized chemicals were tested in the PZ-TR assay, AZ-AhR assay, TTR assay and series of TPO assays on several models.
- Many common pollutants such as bisphenol A, triclosan, and benzophenone 2 can influence multiple MIEs (different levels of the TH system) in the thyroid hormone regulation.
- TTR-binding, critical for the transport of TH to the target tissues is often affected at the lowest concentrations – frequently the most sensitive MIE.

Table 1 EC50 values of prioritised chemicals in AZ-AhR assay, PZ-TR assay, TPO assay and T4-TTR assay. NA means no EC50 could be derived due to no low or no effect. An asterisk* indicates that EC20 levels, EC – agonistic effect, IC – antagonistic effect. More high colour depth means that more influence MIEs were found.

Abbrev.	Chemical	AhR	TR	antiTR	TTR	hTPO	rTPO
		EC50 (uM)	EC50 (uM)	IC50 (uM)	IC50 (uM)	IC50 (uM)	IC50 (uM)
BPA	**2,2-Bis(4-hydroxyphenyl)propane (Bisphenol A)**	na	**44.3**	na	**57.6**	**120.4**	**142.9**
TCS	**Triclosan**	na	**16.6**	na	**2.71**	**62.5**	**77.5**
BP2	**2,2′-4,4′-tetrahydroxy benzophenone**	na	**30.3**	na	**0.24**	**38.5**	**29.6**
RSC	Resorcinol	na	na	**1.75**	na	**13.84**	**15.73**
T3	3,3′,5-Triiodo-L-thyronine	na	**1.26**	na	**0.195**	na	na
T4	3,3′,5,5″-Tetraiodo-L-thyronine	na	**0.08**	na	**0.077**	na	na
DBP	Dibutylphthalate	na	**203**	na	**241.1**	na	na
IOP	Iopanoic acid	na	**110.7***	na	**19.1**	na	na
TPP	Triphenylphosphate	na	**23.2***	na	**403.4**	na	na
PTU	6-propylthiouracil	na	na	na	na	**11.7**	**13.2**
MMI	Methimazole	na	na	na	na	**3.91**	**3.69**
PFOS	Perfluorooctane sulfonate	na	na	na	**0.47**	na	na
PFOA	Perfluorooctanoic acid	na	na	na	**0.83**	na	na
TDCPP	Tris(1,3-dichloro-2-propyl) phosphate	na	na	**0.41**	na	na	na
PCL	Perchlorate	na	na	na	na	na	na
CBZ	Carbamazepine	na	na	na	na	na	na
DON	Deoxynivalenol	na	na	na	na	na	na
AMP	Ampicilin	na	na	na	na	na	na
SA	Salicylic acid	na	na	na	na	na	na
ETU	Ethylene thiourea	na	na	na	na	na	na
SMX	Sulfamethoxazol	na	na	na	na	na	na

Ref: Noyes, P. D. et al. (2019). Evaluating chemicals for thyroid disruption: Opportunities and challenges with in vitro testing and adverse outcome pathway approaches. Environmental health perspectives, 127(9), 095001.

Street, M. E. et al. (2018). Current knowledge on endocrine disrupting chemicals (EDCs) from animal biology to humans, from pregnancy to adulthood: highlights from a national Italian meeting." International Journal of Molecular Sciences 19.6 : 1647.